SCIENCE

FRIDAY, JUNE 27, 1919

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MSS. intended for publication and books, etc., intended for review should be sent to The Editor of Science, Garrison-on-Hudson, N. Y.

GROWTH IN ORGANISMS[1]

THE main proposals discussed in this address were as follows:

1. The development of an organism from the spore or embryonic stage includes the two processes of auxesis or enlargement and of differentiation both in the single cells or elements and in the organs.

2. The present studies are based upon the conception that living matter is composed mainly of pentosans and albumins or albumin derivatives with lipins as a minor component. The proportion of the main components may vary from nearly a hundred per cent. to nearly zero.

3. The principal and characteristic substances of the two groups are practically non-diffusible and hence come together only as an intimate mixture in a colloidal condition, with varying arrangement.

4. Growth of living matter consists of hydration with accompanying swelling and of accretion of solid matter, the two processes being actually independent.

5. The hydration of the substances belonging to the two main components is affected in an opposite manner by hydrogen ions, and is variously modified by temperature and other conditions: the rate and amount of growth is a resultant of several reactions.

6. Accretions of new material include the absorption of salts which tend to restrict hydration and the incorporation of amino-compounds. So-called nutrient salts do not constitute food but may act as catalysts or releasers of energy in other substances and as controls.

7. The enlargement of cells is almost entirely by the swelling which results from hy-

[1] Presidential address, Pacific Division of the American Association for the Advancement of Science meeting at Pasadena, June 19, 1919. Manuscript abbreviated by the author.

dration in their earlier stages, and later the enlargement of the syneretic cavities in the colloidal structure is followed by the distending or stretching action of osmostic pressures in the vacuoles thus formed.

8. Illustrations by records of growth of leafy stems, joints of cacti, fruits of *Solanum* and trunks of trees.

The development of an organism from the single- or few-celled stage to the stature of the adult individual is generally characterized as growth. One of the first facts that comes to the notice of the observer who follows the life history of an animal or plant from the egg or the spore or from the resting stage in a seed to maturity is that all parts of the individual do not enlarge at the same rate, and that if attention be fixed upon the most readily available object for such a study, the root or shoot of any plant, it will be seen that the power of expansion seems to reside only in the region of the tip in the case of the root and in the tip and in certain regions in the younger internodes of stems, while such organs as the leaves of grass elongate by the action of a growing zone at the bases. There are of course many specializations of this action such as those displayed by simple organisms in which a single cell is the inidvidual and when this reaches full size all possible growth is accomplished. As our principal purpose in the present discussion is to present the action of the protoplasm in growth it has been found most convenient to use facts discovered by the measurement and analyses of plants consisting of many millions of cells.

With magnifications of much less than a hundred, we readily see that the embryonic cells of a plant which may be imagined as of a cubical or prismatic form and consisting of a dense mass of colloidal matter, become larger, that they also change form, show new structures in the mass and that the enclosing wall takes on a variety of forms. These changes determine the final part which the maturing cell may play in the complex processes of the organism. The architecture of the plant includes many beautiful mechanical designs and it would be well to guard against the error of considering it as simply a set of sacs, test tubes, and bits of jelly by recalling the fact that it is, like all living things, an engine which not only picks up its fuel, manufactures it into briquettes, or their physiological equivalent, burns this fuel, the derived energy being used in a variety of ways, but while this is going on the machine is also adding to, repairing and altering its own parts. This, however, does not imply that any special or mysterious "life forces" are concerned. The physiologist may in fact identify a large number of the things that may happen in the cell and he may imitate many of them and the progress of science will be marked by the successive subjugation of others, but to assemble the material in a way to obtain the complexity and the sequences of reactions of living matter is beyond our capacity for manipulation, and our failure may not be ascribed to the lack of any elusive vital spark.

The taste for polysyllabic definitions of protoplasm has waned and we are not so much concerned with inclusive descriptions as with an understanding of the nature of the substances which enter into its composition and how these react when subjected to conditions which may prevail in the cell. Protoplasm when viewed with a low power microscope appears to be a silvery translucent mass of material like a highly hydrated jelly, which, in fact it really is, being composed of about one to two parts of solid matter to about two hundred of water. The constituency of the solid part, or the residue which is obtained by driving off all of the water is a matter of no little interest, since it is upon this physical basis that all of the properties of the organism rest.

The proteins or albumins are invariably present, and the transformations in the highly complex molecules of the nitrogenous compounds in living matter offer some tremendous difficulties in interpretation and at the same time yield the material for some of the most romantic chapters in biological science. Present in every cell, these substances may not move from one protoplast to another ex-

cept in the highly hydrated state known as peptones, or when broken apart into comparatively simple amino-compounds. Gelatine, a substance of an albuminous character, has been widely used in experimental work which had for its purpose the determination of the properties of living matter, but we are now so far advanced as to know that it may represent the qualities of the protoplast only in so far as these may be identical with amphoteric compounds. In other words, the behavior of gelatine may be used to some extent to simulate the reactions of protoplasm which consists largely of albuminous substances. This is not a universal condition and in fact is the exception in plants.

Lipins or fatty substances form an important part of the living matter of animals and in their growing cells may constitute as much as two per cent. of the solid matter, amounting to one part in a thousand of the total weight. The lipins may unite with phosphoric acid, with carbohydrates, or with nitrogenous substances such as the amino-acids; giving diverse materials, the action of which in the life processes is but dimly comprehended.

The physiologist who devotes himself to the study of life as exemplified by animal forms deals with a protoplasm in which the proteins and lipins predominate, and is excusably apt to believe in the universality of the properties he uncovers by a study of their reactions. The presence of mucin, gums and mucilages in living matter has long been known, but the determination of their definite occurrence as a component part of the mechanism of the cell was first acomplished at the Desert Laboratory. Numerous analyses show that the pentoses and their condensation products the pentosans are abundant in plant cells, and that they may form a larger proportion of its dry weight than do the proteins or nitrogenous substances.

Here however, we must avoid the mistakes of our predecessors by assuming a universal condition. Specialized organs or cells, eggs, spores, pollen cells, etc., may have a protoplasm in which the protein material may make up almost the entire solid matter, and at the same time it is not to be assumed that the main components are evenly distributed throughout the mass of the protoplast, as it is very well known that the nucleus and other special organs of the cell are high in albumins. Consultation of available information on this point shows that in bacteria for example over 90 per cent. of the solid matter may be albuminous. The analyses of cacti made by Dr. H. A. Spoehr at the Desert Laboratory show that not more than a tenth of the living matter is proteinaceous, and that the greater part of the cell content is carbohydrate, pentosans, of which gum arabic, tragacanth, mucilage and agar are common examples, these being in fact combinations of the simpler pentose and hexose sugars.

Miss Stewart of Barnard College has recently described the manner in which pentosans formed in the cytoplasm accumulate in a layer next the wall leading some observers to believe mistakenly that they were formd by the hydrolysis of wall material. In other cases masses were formed in cavities in the protoplasm. Gross chemical analyses determine the presence of such substances in material in which they occur only in finely divided form in the colloidal mixture and may not be detected by microchemical methods. At present our knowledge of these substances is confined chiefly to their action as a part of the hydration or growth mechanism, and it is by no means clear that they are not more or less included in the metabolic cycle.

These statements are not to be taken as implying a simple composition for protoplasm: The different and various pentosans on the one hand and the amino-compounds built up by the plant or derived from albumins have various special characters although the first agree in being weak acids, and the second are amphoteric, capable of acting as either acids or bases according to conditions. As an example of these differences there has been much discussion as to whether or not protoplasm was soluble or miscible in water. It is obvious that living matter in which the pentosan was a mucilage like gum arabic would be miscible with water, while a pentosan like

tragacanth would be less soluble, and a group like agar, for example, would not appear to be soluble at all. It is by no means implied that solubility invariably depends upon differences in the carbohydrate component as it might also result from the character of the amino-compounds or proteins present, expecially in a protoplasm rich in nitrogen.

My studies of growth have been carried out on the assumption that the principal features of importance are those which might be due to the reactions of the carbohydrates and of the proteins which may be present. It is in order therefore to inquire into the condition in which these substances may occur in living matter particularly with respect to their relation to each other. The first and most important relation to be considered is the fact that the mucilages or pentosans and the albumins of amino-compounds of the cell may diffuse into each other very slowly or not at all. Their joint presence in living matter is in a condition in which they are intimately mixed in a colloidal condition. Molecules or groups of molecules of each lie side by side with various possible arrangements. Thus it is conceivable that the mucilage of a cell might be in the form of a mesh or honeycomb with the proteins forming droplets enclosed in the continuous structure, or the reverse might be the case; again substances of both groups might each form a continuous meshwork interlocking with the other, and another category of variables would be introduced by the lipins which might be interposed or incorporated in these systems. Living matter probably does not remain fixed in any one of these simple arrangements, or in any one of a dozen others which might be described if space permitted, and the suggestion is ventured that the play of molecular force where aggregates of a different kind are in contact may constitute the essential and characteristic action of living matter.

Let us now fasten attention upon the theoretical final structure of protoplasm and endeavor to construct for ourselves a mode or plan of action which might be followed in its growth. Growth as has been defined consists of two processes. First the molecules or aggregates of molecules of the two kinds, the carbohydrates and the albumins, combine with and absorb water, thus increasing the volume of these units regardless of whether such molecules be in the form of droplets or fibrillæ of a meshwork. Instances of growth are known in which water only has been added to the colloidal structure in which in all probability the solid particles have been variously rearranged. In general however growth is accompanied by the accretion of molecules of solid material in such manner that as development proceeds their proportion to that of the water taken increases and organs are then said to show an increase of relative dry weight with age.

On the other hand, my own studies have shown that succulent organs or stems, such as leaves of the Crassulaceæ, joints of cacti, fruits, etc., do not show such increase and the proportion of solid matter and of water undergo but little change, their incorporation being at a rate which keeps them near the initial proportion. It is suggested that such action may be shown by the fleshy fungi although I have not seen any data bearing directly upon this matter.

The conditions under which hydration may ensue are by no means identical for the two main constituents of living matter. Thus the albumins and their derivatives as exemplified by the behavior of gelatine show a swelling determined or facilitated by the hydrogen ion concentration or acidity of the solutions, being increased as this rises. The pentosans, on the other hand, show no such increase, and being weak acids, their hydration is retarded by the hydrogen ion. The swelling of a mixture of the two will therefore be a resultant of these effects and of the proportion of the two elements in the living mixture, and as the unceasing action of respiratory metabolism results in the formation of some residues of acids, the condition of hydration of any mass of protoplasm may be said to reach a volume determined by these opposed reactions. The effects in question may be illustrated by the citation of my experiments, in which gelatine

was found to show a swelling in hundredth normal acetic acid fifty per cent. greater than in distilled water, agar forty per cent. less, and a combination of eight parts of agar and two parts of albumin, about forty per cent. less than in water.

The hydrogen ion concentration of the fluids in a plant cell are controlled by the buffer conditions which exist there, but still the range of variation is much wider than that found in the circulatory systems of animals. Bases or cations are seen to affect the swelling of the plasmatic mixtures in my experiments. Various authors having secured results indicative of accelerating effects of certain amino-compounds on growth, some swelling tests of the effects of these substances were made with the discovery that such an amino-acid as glycocoll in hundredth molar solutions seems to retard the swelling of gelatine, at least when the increase in thin dried plates is considered, and to accelerate the swelling of agar to and beyond the total in water. The mixture of agar and albumin, as well as a mixture of agar and gelatine, shows a greater hydration in glycocoll than in water.

The possible physiological significance of these results is heightened by the knowledge of the fact that some of these amino-compounds may be taken to be universally present in growing cells and they probably vary less than the organic acids. It is suggested that the ammonia group in these compounds may form a salt with the carbohydrates with the effect of increasing the hydration capacity. Whether any reaction with, or effect upon, the hydration of the protein element occurs is not yet clear, although it is obvious that such action might be of fundamental importance in nutritive metabolism. The entire matter of hydration may be briefly summarized by the statement that the fundamental properties of a colloidal mixture or of living matter will depend upon the proportion of albumins and of pentosans, and upon the properties of the particular substances of each group which may be present. Hydrogen ions within the possible range of concentration increases hydration of the albuminous substances and depress that of the pentosans. Bases or cations exert a reverse effect on the albuminous substances and depress hydration of the pentosans slightly. Certain amino-compounds depress the swelling of albuminous compounds, but facilitate the hydration of pentosans and sections of such substances when mixed in a proportion of four to one with albumin undergo hydration to a degree equivalent to or even greater than that in water.

The second phase of growth, that of the incorporation of molecules of solid matter is not so easily described since it is not so directly susceptible of experimental test. If the conception of the pentosan-albumin composition of protoplasm is correct, it is obvious that the mass of living matter may not be increased simply by the addition or diffusion of sugars into the meshwork, as is supposed by some writers.

Before the material in these carbohydrates may actually become a part of the colloidal living mesh it is undoubtedly broken down to some extent by enzymatic or respiratory action, part of the material being carried through transformations to organic acids or carbon dioxide, some of the material is combined with the ammonia group (NH_2) to form amino-compounds, some with the lipins, while some of these sugars may be converted to the pentose form in which they would so markedly affect the hydration capacity of the mass.

By way of crude illustration, protoplasm might be regarded as the wick of a lamp which draws sugar into its meshes, burns the sugar and in the burning some of the sugar not completely consumed unites with other substances to form additional fibers of the wick.

At this point it would be well to divert attention for the moment to the so-called "nutrient" salts, the presence of which in the soil and in the liquids of the plant is so indispensable to the plant. It is necessary for an understanding of the real nature of growth to have clearly in mind that living matter is a colloidal mixture of proteins and carbohydrates, which takes up water and gains solid material in growth by processes which are actually retarded by these salts. These com-

pounds in fact yield no energy and furnish no building material. They may act as catalyzers or as releasing agents, and as controls of water absorption or as guides in colloidal arrangement, but they are not "food-material" in any sense. The constituents of fertilizers should be designated as "culture salts" and as such have all of the importance which has been imputed to them; a determination of the composition and proportion of salts in a culture solution which will induce maximum production of grain, fruit or forage is a problem of the first rank now happily receiving something like an adequate investigation.

The foregoing suffices to account for the mechanics of growth or expansion of a single-celled or naked organism. The development of complex, massive or higher organisms especially in plants, however, is accompanied by the formation or deposition of an outer layer of denser consistency which occurs at any phase boundary of colloidal material. This membrane so-called is in any case a product of the surface energy of the mass or system of living material in the cell and of the material in contact and its constitution, and even its structure must vary as widely as that of the protoplasm which produces it.

External to the membrane is the cell-wall which begins to be formed around plant cells as soon as they divide or are separated and this wall increases in rigidity and offers greater resistance to stretching as it grows older.

The arrangement in question, therefore, is one in which the expanding and growing protoplast is enclosed in a sac or bag of its own making and which acts as a screen not only in allowing some materials to pass while others are shut out, but also is so constructed that some solutions pass through it more readily into the cell than out of it, these being simply examples of some of the many facts discussed under the designation of permeability. The external screening membrane takes on a special significance in connection with the osmotic action of the vacuoles.

These sacs were at one time thought to have a morphological value, but it is now understood that almost any hydrating colloidal mass may exhibit syneresis in which cavities or canals are formed in which the colloidal material accumulates in an attenuated or liquid condition. These syneretic cavities increase by absorption of water and by the time the protoplasm of the cell has attained about half of its ultimate bulk in some instances, these cavities have enlarged to occupy a space as large as the protoplasm and acting as vacuoles by which they are ordinarily known, eventually fill a much larger space. The expansion of these vacuoles and the consequent increase in volume of the cell constitutes part of the enlarging action of growth, and this expansion takes place by the force of osmotic action. and the result of such stretching is to set up a tension ordinarily designated as turgidity. The vacuoles continue to hold some of the colloidal material and may also carry in solution almost any substance in the cell which may be passed into them by osmosis or diffusion, including sugars, salts, acids, amino-compounds, etc.

The enlargement of the individual masses of living cells in organisms entails a certain amount of work which in the earlier stages is derived almost entirely from imbibition or adsorption, and while such action continues throughout the growth or life of the living matter, there is in addition the stretching action exerted by the expanding vacuoles by osmotic action. The growing regions or plants at all times include cells in all of these stages, from the newly separated protoplasm which is expanding entirely by imbibition of water and incorporation of new material, others in which the synereticaily formed vacuoles are increasing and thus adding to the volume of the cell by osmotic action, and others approaching maturity in which the vacuole may have attained such size as to occupy many times the space of the living matter which may indeed now be but a sac with its layers of irregular thickness lying internal to the wall, which now has become dense and rigid.

The measurement of the growth of a stem, root or fruit of a plant will, therefore, show the composite changes in volume of cell masses

in all of these stages, and consequently express the action of imbibition and osmosis.

The distinct action of imbibition and the later joint action of hydration by osmosis and by imbibition may be most readily recognized, in organs in which the region of growth is generalized as in the ovate flattened joints of *Opuntia* or in such globular fruits as the tomato. The measurement of the growth of one of these joints may be begun when it has a lateral area no larger than the thumbnail, and during this stage the increase is rapid and shows a minimum disturbance from changes in external conditions, as shown by the illustrations. Growth continues throughout the entire mass until an advanced stage of development is reached, when it first slackens in the basal portion. By this time large vacuoles have been formed in the thin-walled cells, and water loss from the surfaces of the organ has reached such a rate that great daily variation in the volume results and actual shrinkage may ensue. A similar history may be predicated for such structures as the large berry-like fruit of the tomato, it being noted that the material in both illustrations takes on solid matter and water at such rate that not much alteration in their proportions occurs during development.

The enlargement of the trunk of a tree results from the multiplication and growth of cambium and other cells on the outside of the trunk directly inside and covered by the bark. The trunk of the tree is in effect a cylinder of moist but dead woody tissue surrounded by a living sheath which becomes very active at some time in the year and which as a result forms an additional layer or sheet of wood on the trunk which in cross section gives the appearance which has caused it to be designated as an annual ring of growth.

The actual course of growth or formation of these annual cylinders or, more strictly speaking, cones, has not until recently been measured. In 1918 I was successful in making a working model of a dendrograph which might be attached to the trunk of a tree in such manner that its changes in volume due to whatever causes were traced on a ruled sheet of paper carried by a revolving drum. The essential part of this apparatus is a yoke of metal, which has two bearing screws resting on the trunk and carrying a third contact point on the end of the pen lever. It was not possible to make a practicable instrument until a yoke could be constructed which showed but little variation as a result of changes in temperature. Three alloys with a very low temperature coefficient, bario C., manganin and invar have been used and dendrographs are now in operation on the trunks of two species of pine, and oak, an ash, a sycamore and a beech tree, and as these instruments were placed in position before growth began in 1919, there is every prospect that seasonal records will be obtained from which the principal features of growth may be seen. Weekly records show that these trees do not behave alike and that many conditions are to be considered in interpreting the records.

It is evident for example that but little is known concerning the properties of bark as a water-proofing or protecting coat for the tree. The loose bark of the ash and pine trees seems to allow such a great water loss from the surface during the mid-day period as to cause actual shrinkage which does not occur in trees such as the beech and live-oak, which have a perfect living green outer bark or skin. The facts disclosed by these records can not fail to be of interest in a discussion of any phase of the complicated problem of the ascent of sap.

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AMERICAN geology has lost one of its foremost leaders, one who promised to stand as high as the highest. Professor Barrell's other colleagues will undoubtedly agree with Professor T. C. Chamberlin when he says: "We had come to look upon him as one of the most promising leaders in the deeper problems of earth science. We feel that his early departure is a very sad loss to our profession not only, but to the whole group of sciences that center in the earth and its constitution."